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Immunization of Cattle with a Variant-Specific Surface Antigen of *Trypanosoma brucei*: Influence of Different Adjuvants†

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Nine different adjuvants were examined for their ability to potentiate the humoral and cell-mediated immune responses of cattle to a soluble glycoprotein antigen prepared from *Trypanosoma brucei*. Serological responses as measured by the Farr assay were best augmented by the oil-based adjuvants and saponin. Cell-mediated immunity, as assessed by specific lymphocyte transformation in vitro, was enhanced by all oil-based adjuvants at different intervals after immunization. Results from a challenge infection of immunized cattle with the homologous clone of *T. brucei* and from neutralization tests indicated that protection against infection was better correlated with specific antibodies than with cell-mediated responses. From these considerations, and the absence of tissue reactions at the site of inoculation, saponin was considered more practical than the oil-based or bacterial adjuvants for the elicitation in cattle of antibodies to purified soluble antigens.

Practical immunization, particularly with inactivated antigens, often requires the use of adjuvants to potentiate the immune response. Despite the use of adjuvants in several commercially available vaccines against infectious diseases of cattle, only a limited number of studies have examined the ability of adjuvants to enhance immune responses in cattle. The antigens used have been largely of bacterial and viral origin. In particular, the use of adjuvants in enhancing the immune response to foot-and-mouth disease virus has been studied in some detail, and the relative merits of aluminum hydroxide, oil, and saponin adjuvants in this system have been evaluated (9, 14). However, with the availability of several new adjuvants, it appeared to us that a more comprehensive understanding of the ability of a range of adjuvants to potentiate different types of immunological responses in cattle was required.

This paper describes two experiments designed to investigate the effects of different adjuvants on the humoral and cellular immune responses of cattle to the soluble variant-specific antigen (VSSA) of *Trypanosoma brucei*. This antigen is a glycoprotein of approximate molecular weight 60,000 (8) and constitutes the major component of the surface coat of the trypanosome. During the process of antigenic variation

in trypanosomes, the VSSA changes; however, it has been shown in mice that immunization with VSSA protects against challenge with trypanosomes bearing the same VSSA (8). Thus, VSSA, as an antigen, is biochemically well characterized and is available in a relatively pure form, and antibody and cell-mediated immune responses to it are readily measured; furthermore, the protective effect of immunization can be tested by challenging with trypanosomes of the same variable antigen type. Nine adjuvant preparations were tested for their ability to potentiate antibody and cell-mediated immune responses to VSSA and to induce protective immunity. These investigations also provided the opportunity to evaluate the relative importance of humoral and cell-mediated immune responses in immunity to trypanosomal infections in cattle.

MATERIALS AND METHODS

Animals and housing. The cattle used were castrated males of the Boran breed aged between 9 and 12 months and were obtained from an area of Kenya where trypanosomiasis does not occur. Examination of their sera by indirect immunofluorescence, using Formalin-fixed *T. brucei*, *T. congolense*, and *T. vivax* as antigens, failed to show evidence of previous infection. In each experiment, before challenge with trypanosomes the cattle were yarded as one group. However, at the time of challenge and during the subsequent period while parasitemia was being moni-

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tored, the infected cattle were housed in an insect-proof cattle shed. Throughout the experiment the animals were allowed free access to hay and water.

Trypanosomes. (i) **Trypanosomal antigen for immunization and assay of antibody responses.** VSSA was prepared from the clone of *T. brucei* known as ILTat 1.3, which is derived from *T. brucei* stock UHFMB0/64EATRO/795, isolated in 1964 from a cow in Kenya and previously designated 227 after a series of passages in mice. VSSA was isolated from trypanosomes grown in lethally irradiated rats by the method described by Cross (8).

(ii) **Trypanosomal antigen for in vitro lymphocyte transformation assay.** Trypanosomal antigen for addition to in vitro cultures of peripheral blood leukocytes (PBL) was prepared by ultrasonication of live trypanosomes as described previously by Emery et al. (11). In addition to *T. brucei* ILTat 1.3, an unrelated clone of *T. brucei* (055) and an isolate of *T. congolense* (GUTR37) were also used for preparation of antigen to test the specificity of the responses. The origin of these organisms has been described previously (11, 18).

(iii) **Trypanosomes for challenge after immunization.** In both experiments, all immunized animals were challenged with live trypanosomes of the homologous ILTat 1.3 clone approximately 6 weeks after immunization. A stabulate of the organisms was inoculated into lethally irradiated rats, and 3 days later the animals were bled out into heparinized containers. Blood containing the organisms was then diluted to the appropriate volume in phosphate-saline-glucose (PSG), pH 8, and inoculated intravenously. Cattle in the first experiment received 10^5 viable organisms; those in the second were given 10^4 organisms.

Experimental design. We examined a variety of adjuvants for which activity had been documented in cattle or in other species. Muramyl dipeptide (MDP), which is considered to be the minimal structure capable of replacing mycobacteria in complete Freund adjuvant (CFA) (10) and which has been shown to have considerable potential as an adjuvant in aqueous solution (6), was incorporated into incomplete Freund adjuvant (IFA), and its adjuvanticity was compared with that of both IFA and CFA. Two bacterial adjuvants, *Propionibacterium acnes* (formerly designated *Corynebacterium parvum*) and *Bordetella pertussis*, were also examined. In addition to their adjuvant properties, these agents when given alone have been shown to increase the nonspecific resistance of mice to *Plasmodium* (7) and to trypanosomes (15). The synthetic polynucleotide polyadenylate-polyuridyate [poly(A-U)] was found by Casavant and Youmans (5) to enhance the immune response of guinea pigs to the purified protein derivative of *Mycobacterium tuberculosis*. Saponin had been used previously in cattle to boost serological responses to Formalin-inactivated foot-and-mouth disease virus (14). More recently, adsorption of bacterial antigen onto aluminum hydroxide gel (AH) with subsequent incorporation of the preparation into IFA elicited a better specific antibody response in sheep than an aqueous solution of antigen administered in IFA alone (20).

In the first experiment, 27 cattle were allocated into 5 groups. Cattle from three of these groups were immunized with antigen plus adjuvant, one group received antigen in PSG, and the remaining group

served as challenge controls. In the second experiment, 40 cattle were divided into 9 groups, each comprising 4 or 5 animals. The cattle in seven of these groups were immunized with preparations of antigen and adjuvant. A further group of unimmunized animals was used as controls for measurement of serological and cell-mediated immune responses after immunization and challenge of the other groups; the animals of the remaining group served as challenge controls. In each experiment, one group of cattle was immunized with VSSA in IFA to provide comparability between the experiments. In both experiments, a total of 200 μ g of VSSA was incorporated into each adjuvant-antigen preparation so that the final concentration of antigen was 50 μ g/ml. With each preparation, a total of 4 ml was injected intramuscularly at a depth of approximately 4 cm on the left side of the neck about 25 cm behind the ear. During the course of immunization and subsequent challenge, blood samples were collected at intervals for serum, for isolation of leukocytes, and for monitoring parasitemia. Animals in the first experiment were challenged with viable trypanosomes 43 days after immunization, and cattle in the second experiment were challenged with viable trypanosomes 46 days after immunization.

Experiment 1. The adjuvant-antigen preparations used for immunization were: (i) a solution of VSSA at 100 μ g/ml in PSG emulsified in an equal volume of IFA consisting of Bayol F (Esso Petroleum Co. Ltd.) containing 10% (vol/vol) Arlacel A (Sigma Chemical Co., St. Louis, Mo.); (ii) VSSA adsorbed at an optimal dilution onto AH (Alhydrogel, Superfos, Copenhagen, Denmark) and diluted with PSG to a concentration of 50 μ g of VSSA per ml; and (iii) VSSA adsorbed onto AH and emulsified in an equal volume of IFA (AHO) as described previously (20).

Experiment 2. The adjuvant-antigen preparations used for immunization were: (i) a solution of VSSA at 100 μ g/ml in PSG incorporated in IFA as used in experiment 1; (ii) a solution of VSSA at 100 μ g/ml in PSG containing killed *M. tuberculosis* emulsified in an equal volume of IFA to constitute CFA (the concentration of *M. tuberculosis* was such that each animal received a dose of 2 mg in the inoculum); (iii) a solution of VSSA at 100 μ g/ml in PSG containing poly(A-U) (Miles Laboratories Ltd., Slough, England) emulsified in an equal volume of IFA [each dose of inoculum contained 2 mg of poly(A-U)]; (iv) a solution of VSSA at 100 μ g/ml in PSG containing *N*-acetylmuramyl-L-alanyl-D-isoglutamine (MDP; Pasteur Institute Production, Paris, France) emulsified in an equal volume of IFA (each dose of inoculum contained 2 mg of MDP); (v) a solution of VSSA at 50 μ g/ml containing killed *P. acnes* (Wellcome Research Laboratories, Beckenham, England) at a concentration of 1.25 mg/ml; (vi) a solution of VSSA at 50 μ g/ml containing killed *B. pertussis* (Wellcome Research Laboratories) at a concentration of 1.25 mg/ml; and (vii) a solution of VSSA at 50 μ g/ml containing saponin (saponin powder P.3, batch 62020; Food Industries Ltd., Bromborough, England) at a concentration of 1.25 mg/ml.

Examination of blood samples for parasitemia. Jugular blood samples were collected from the cattle for 3 days before and 14 days after challenge with trypanosomes. These were examined for the presence of parasites by using the blood buffy coat dark-ground phase technique described by Murray et al. (16). On

the basis of the scoring system for parasitemia reported previously, samples with a score of 4+ or greater were diluted and counted in a hemacytometer (17).

Serology. Serum antibody titers to *T. brucei* clone ILTat 1.3 were estimated by means of (i) a Farr assay and (ii) a serum neutralization test.

(i) **Farr assay.** The measurement of VSSA binding capacity (ABC) was performed according to the method of Brownstone et al. (4), the principal deviation being that the volumes of serum and antigen used were reduced from 500 to 20 μ l. Dilutions of test serum were made in 10% normal bovine serum in 0.1 M borate buffer, pH 8.0. VSSA was iodinated according to the method of Bolton and Hunter (1); labeled antigen was added to each dilution of serum in duplicate, the final concentration of the antigen being 10^{-7} M containing 20,000 trichloroacetic acid-precipitable cpm added per tube. After incubation overnight at 4°C, 40 μ l of saturated ammonium sulfate was added to each tube and then mixed and placed on ice for 5 to 10 min. The tubes were centrifuged at 4°C at $1,800 \times g$ for 20 min, and the supernatants were removed. The precipitates were then counted in a gamma scintillation spectrometer (Packard model 5360). Calculation of ABC made use of the assumptions given in Brownstone et al. (4). However, based on a preliminary binding curve, the point at which antigen binding ceased to be proportional to antibody concentration and became proportional to \log_{10} antibody concentration was found to be 30%. Calculation of this figure involved correction for nonspecific binding as described by Minden and Farr (13). Values of antigen binding in excess of 70% and less than 10% were disregarded.

(ii) **Serum neutralization test.** Serial dilutions in PSG of pooled serum samples collected from each group of cattle on the day before challenge with trypanosomes were added to an equal volume of PSG containing *T. brucei* clone ILTat 1.3 at a concentration of 10^4 /ml. After incubation on ice for 30 min, 0.2 ml of each serum dilution and trypanosome mixture was inoculated intraperitoneally into each of eight mice, which were then examined daily for parasites for 30 days.

Lymphocyte transformation. PBL from cattle were prepared at weekly intervals after immunization. In vitro cultures of PBL were established as described by Emery et al. (11) to examine specific proliferative responses to ultrasonicated trypanosomes from *T. brucei* ILTat 1.3, *T. brucei* clone 055, and *T. congolense* GUTR37. The cultures were pulsed with [125 I]deoxyuridine after 90 h, and the cells were harvested after a further 6 h. The stimulation indices (SI) were calculated from the formula: $SI = \text{counts per minute in cultures containing antigen} / \text{counts per minute in cultures without antigen}$.

Pathology. Two animals from each group in both experiments were necropsied 4 to 6 weeks after challenge with trypanosomes, i.e., 10 to 12 weeks after immunization. The site of injection of either antigen alone or adjuvant-antigen preparation was examined. When macroscopic lesions were evident, samples were taken for histopathological examination. Tissues were fixed in mercuric chloride Formol, processed, and sectioned by conventional methods and stained with hematoxylin and eosin.

Statistics. Results were analyzed by the Student *t*-test.

RESULTS

Experiment 1, serology. In experiment 1, antibody activity, as detected by radio-immunoassay, in the serum of cattle in groups receiving VSSA with either IFA or AHO increased rapidly during the 1st week after immunization (Fig. 1). At the end of the 2nd week, the mean log ABCs observed in sera from cattle in these groups were 1.66 and 1.84, respectively. By comparison, mean log ABCs of only 0.39 and 0.91 had been achieved at this time by cattle in groups inoculated with VSSA alone or VSSA with AH. With minor variation, antibody activity in the sera of cattle in all groups was maintained at similar levels until the time of challenge. Statistical analysis indicated that the antibody responses to VSSA with IFA and VSSA with AHO were significantly greater than those induced by VSSA alone or VSSA with AH ($P < 0.01$). After challenge with trypanosomes, an increase in serum antibody was detected in all groups of cattle. Two weeks after challenge, log ABCs were of a similar magnitude in all immunized animals and were higher than in the sera of the cattle in the challenge control group.

Table 1 shows the results of the neutralization assays carried out on pooled sera collected on the day before challenge from the five groups of cattle in experiment 1. Only the pooled sera from the cattle inoculated with VSSA and AHO or VSSA and IFA reduced the infectivity of the trypanosome inoculum, and clearly, pooled sera from the former group were more effective; the dilution of serum which reduced infectivity to 50% was found to be $10^{-2.5}$ for cattle receiving VSSA and AHO and $10^{-0.7}$ for cattle receiving VSSA and IFA.

Lymphocyte transformation responses. No proliferative response was observed when antigen prepared from *T. congolense* GUTR37 was included in cultures of PBL from cattle immunized with the VSSA from *T. brucei* ILTat 1.3. Some degree of proliferation (SIs less than 3.1) was observed in PBL from the immunized cattle when cultured in the presence of antigen prepared from *T. brucei* 055. In comparison, more pronounced proliferative responses (SIs between 2.8 and 4.7) were elicited in these PBL by ultrasonicated *T. brucei* ILTat 1.3. However, only the proliferation responses induced by ultrasonicated *T. brucei* ILTat 1.3 in PBL harvested from cattle 7 days after immunization with VSSA in IFA or AHO were significantly greater ($0.025 < P < 0.05$) than the respective responses of PBL from unimmunized cattle.

Response to challenge with live trypanosomes. Table 2 shows the results of parasitological examinations of blood samples collected from the cattle in experiment 1 after challenge with *T.*

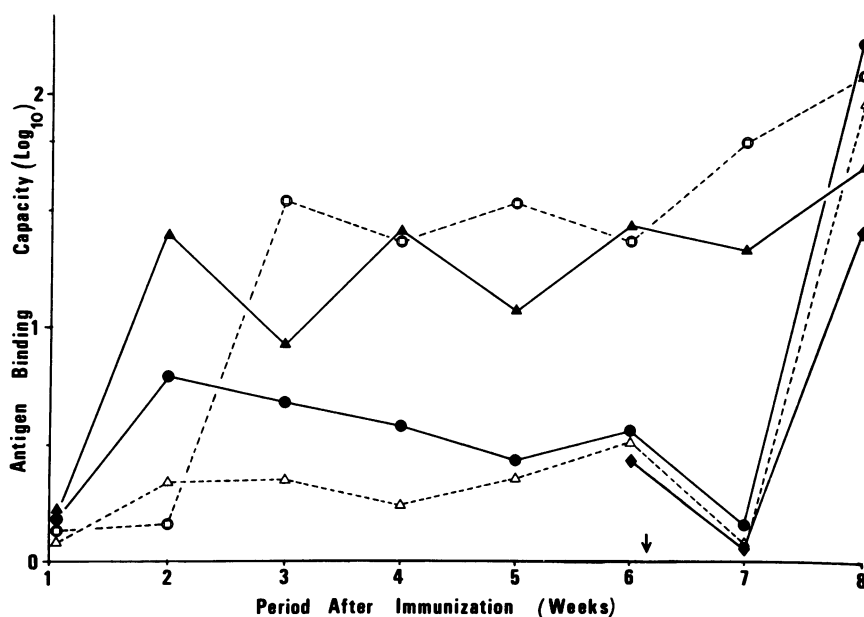


FIG. 1. Mean serum antibody responses to VSSA from *T. brucei* ILTat 1.3 after immunization without adjuvant (Δ) or with AH (\bullet), IFA (\blacktriangle), or AHO (\circ) in experiment 1. Cattle were exposed to challenge infection with 10^5 *T. brucei*. (\downarrow) ILTat 1.3 43 days after immunization. The mean serum antibody response to VSSA from *T. brucei* ILTat 1.3 after infection is also shown for the nonimmunized cattle (\blacklozenge — \blacklozenge).

brucei ILTat 1.3. In cattle given VSSA with either IFA or AHO, there was clearly evidence of a delay in the development of a patent infection. Statistical analysis indicated that the prepatent periods observed in these groups were significantly different from those in both the untreated cattle and those which had received either VSSA or VSSA adsorbed on AH. This delay in the development of a patent infection was also reflected in a delay in the time to when the first peak of parasitemia was detected in the cattle for the same two groups. Again, these groups were significantly different in this respect from the cattle in the untreated group and from those given either VSSA or VSSA adsorbed on AH. However, despite these differences in the time course of the development of the parasit-

emia, the level of parasitemia achieved in the first peak of parasitemia after inoculation with trypanosomes did not differ between the groups.

Experiment 2, serology. The serum antibody response as measured by the Farr assay in cattle given VSSA in IFA was comparable to that observed in experiment 1. Cattle injected with VSSA in CFA produced the greatest serological response, which reached a peak mean after 3 weeks (Fig. 2). The serological responses were of similar magnitude in cattle immunized with VSSA in IFA, VSSA with MDP in IFA, and VSSA with poly(A-U) in IFA. Log serum ABCs in these cattle increased during the 2nd week after immunization, and levels of serum antibody achieved at 2 weeks were maintained until the time of challenge. Cattle given VSSA with

TABLE 1. Experiment 1: results of the neutralization assays on pooled sera collected from each group of cattle before challenge inoculation with trypanosomes

Serum pools from cattle given:	% of mice infected at dilutions of serum pools					Dilution of serum pool conferring protection to 50% of mice
	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	
VSSA + AHO	0	0	50	100	100	$10^{-2.5}$
VSSA + IFA	0	88	88	88	75	$10^{-0.7}$
VSSA + AH	100	100	88	75	100	— ^a
VSSA	100	88	75	88	100	—
Untreated	100	100	75	88	88	—

^a No protection with undiluted serum.

TABLE 2. Results of parasitological examinations of blood samples collected after challenge infection

Treatment group	Mean prepatent period (days) ^a	Mean time to first peak of parasitemia (days) ^a
Immunized with VSSA and AHO	5.6 ± 0.5 ^b	7.8 ± 0.4 ^b
Immunized with VSSA and IFA	6.0 ± 0.7 ^b	8.2 ± 0.4 ^b
Immunized with VSSA and AH	3.7 ± 0.8	6.0 ± 0.5
Immunized with VSSA	4 ± 0	5.8 ± 0.4
Nonimmunized	4 ± 0	6.0 ± 0.8

^a Mean ± standard deviation of the sample.^b Significantly different from nonimmunized cattle ($P < 0.01$).

saponin showed a similar but lesser initial response which declined slightly 4 or 5 weeks after injection. The poorest response observed with VSSA and *P. acnes* was of the same order as that achieved with VSSA when given without adjuvant in experiment 1. Although slightly greater than with *P. acnes*, VSSA injected with *B. pertussis* resulted in a relatively poor serological response. After challenge with trypanosomes, only those groups which became infected exhibited an increase in antibody.

Table 3 shows the results of the neutralization assays carried out on pooled sera collected before challenge from the eight groups of cattle in experiment 2. Cattle immunized with VSSA and *P. acnes* produced no detectable neutralizing antibodies. Of the pooled sera from other

immunized groups, serum from the group given VSSA with CFA was most active, and that from the group given VSSA with *B. pertussis* was least effective, in protecting the mice against infection. The dilution of serum pools conferring protection to 50% of the mice was similar for sera from groups inoculated with VSSA given with IFA, MDP in IFA, poly(A-U) in IFA, and saponin.

On the basis of the parasitological data obtained after exposure to challenge, it was possible to reexamine sera from cattle in the three groups where less than complete protection was achieved, namely, those given VSSA with CFA, poly(A-U) in IFA, and *B. pertussis*. Within each of the three groups, separate serum pools from those animals which were resistant to challenge

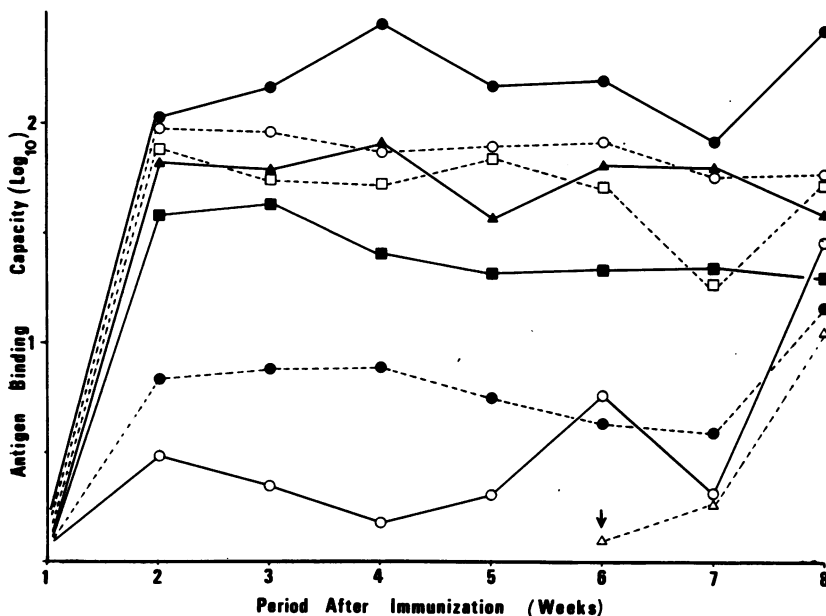


FIG. 2. Mean serum antibody responses to VSSA from *T. brucei* ILTat 1.3 after immunization of cattle, using IFA (▲), CFA (●), MDP in IFA (○), poly(A-U) in IFA (□), *P. acnes* (○), *B. pertussis* (●), or saponin (■) as adjuvant in experiment 2. Cattle were exposed to challenge infection with 10^4 *T. brucei* ILTat 1.3 46 days after immunization (↓). The mean serum antibody response to VSSA from *T. brucei* ILTat 1.3 after infection is also shown for the nonimmunized cattle (Δ).

TABLE 3. Results of the neutralization assays on pooled sera collected from each group of cattle before challenge inoculation with trypanosomes

Serum pools from cattle given:	% of mice infected at dilutions of serum pools				Dilution of serum pool conferring protection to 50% of mice
	10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	
VSSA + IFA	0	0	75	100	10 ^{-1.7}
VSSA + CFA	13	0	0	100	10 ^{-2.4}
VSSA + MDP + IFA	0	13	50	100	10 ^{-1.9}
VSSA + Poly A.					
Poly U + IFA	0	0	100	100	10 ^{-1.5}
VSSA + <i>P. acnes</i>	100	100	100	100	— ^a
VSSA + <i>B. pertussis</i>	0	88	100	100	10 ^{-0.7}
VSSA + saponin	0	13	100	100	10 ^{-1.4}
Untreated	88	100	100	88	—

^a No protection with undiluted serum.

and those which were not were examined for neutralizing activity. In all instances except the group which received VSSA in CFA, those animals which were resistant to challenge exhibited higher serum neutralizing activity than those which were susceptible (Table 4).

Lymphocyte transformation responses. Figure 3 shows the proliferative responses observed in cultures of PBL from cattle in experiment 2 when stimulated with trypanosomal antigens. As in experiment 1, no proliferative responses were observed when antigen from *T. congolense* GUTR37 was included in cultures of PBL from cattle immunized with VSSA from *T. brucei* ILTat 1.3 at some stage after immunization, with the exception of cattle treated with VSSA and saponin (Fig. 3). The most distinct proliferative responses appeared to occur at approximately 2 weeks or 5 to 6 weeks after immunization. In the early phase, PBL from cattle given VSSA and CFA appeared to be most responsive, whereas at the later stage, cultures of PBL from cattle given VSSA in association with MDP and IFA had the highest SIs. There was a tendency of proliferative responses to decline in PBL collected after exposure to challenge infection.

Response to challenge with live trypanosomes.

In the first experiment, in which the cattle received 10⁵ organisms intravenously, none of the immunized animals was completely protected. It was uncertain whether this was due to inadequate immunization or to the fact that the organisms used for challenge were not a homologous population of ILTat 1.3 VAT. Therefore, before challenge of the animals in the second experiment, a neutralization test was carried out in mice on different numbers of organisms, using hyperimmune serum against the isolated VSSA. It was found that the hyperimmune serum, when added in concentrations ranging from 10⁰ to 10⁻² to an equal volume of the trypanosome suspension, was capable of completely neutralizing 10⁴ organisms. However, the neutralization was incomplete with 10⁵ organisms, thus indicating that at the level of 10⁵, the trypanosomes were not a homogeneous population. Accordingly, a challenge of 10⁴ organisms given intravenously was used in the second experiment. Table 5 shows the results of parasitological examinations on blood samples collected from cattle exposed to challenge. In this experiment, complete protection against infection was achieved

TABLE 4. Results of neutralization assays on pooled sera collected before challenge inoculation from cattle grouped according to the combination of VSSA and adjuvant they were treated with and according to their response to challenge inoculation

Serum pools from cattle given:	Response to challenge inoculation	% of mice infected at dilutions of serum pools				Dilution of serum pool conferring protection to 50% mice
		10 ⁰	10 ⁻¹	10 ⁻²	10 ⁻³	
VSSA + CFA	Protected	0	0	0	100	10 ^{-2.7}
VSSA + CFA	Not protected	0	0	0	100	10 ^{-2.7}
VSSA + poly(A-U) + IFA	Protected	0	0	50	100	10 ^{-2.0}
VSSA + poly(A-U) + IFA	Not protected	0	25	87	100	10 ^{-1.6}
VSSA + <i>B. pertussis</i>	Protected	0	0	100	100	10 ^{-1.7}
VSSA + <i>B. pertussis</i>	Not protected	100	87	100	87	— ^a

^a No protection with undiluted serum.

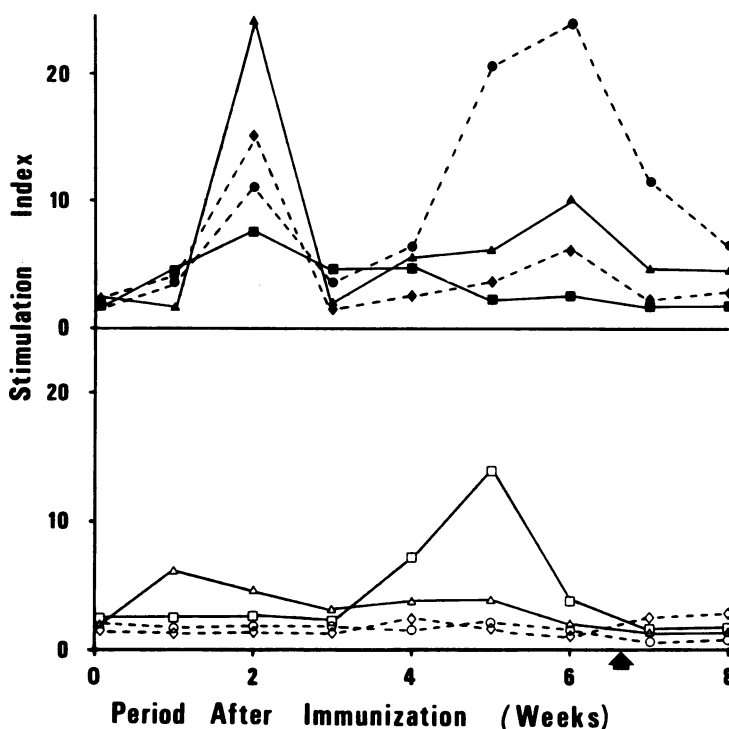


FIG. 3. Mean proliferative responses to *T. brucei* ILTat 1.3 observed in cultures of PBL from cattle immunized with VSSA from *T. brucei* ILTat 1.3, using IFA (■), CFA (▲), MDP in IFA (●), poly (A-U) in IFA (◆), *P. acnes* (□), *B. pertussis* (△), or saponin (○) as adjuvant. Response in control cultures is also shown (◇). Cattle were challenged with 10^4 *T. brucei* ILTat 1.3 46 days after vaccination (▲).

in groups of cattle immunized with VSSA given in association with IFA, MDP and IFA, and saponin. No protection was apparent in cattle given VSSA and *P. acnes*, and partial protection was observed in the three remaining groups.

Pathology. On examination of cattle from all groups at necropsy, a reaction at the site of inoculation was found only in those animals which had received an oil-containing adjuvant, i.e., IFA, CFA, AHO, MDP in IFA, and poly-(A-U) in IFA. The lesions in most instances were found as an irregular plaque in the fascial plane between the muscles of the neck and the lamellar part of the nuchal ligament, usually extending 1 to 2 cm into the muscle. The most marked reaction was observed in animals inoculated with VSSA in CFA; this produced a granulomatous reaction of maximal dimensions 10 cm long, 5 cm wide, and 2 cm in thickness aligned longitudinally in the musculature. Numerous small encapsulated granulomata of variable size up to 7 mm in diameter were found containing white oily material. The other oil-containing adjuvants produced reactions which, although similar in character, were considerably smaller and contained smaller granulomata.

Histologically, the lesions consisted of numerous encapsulated granulomata interspersed in a more diffuse cellular infiltrate. In general, the features of these lesions were similar for all of the oil-containing adjuvants, although with CFA the granulomata were larger and contained large areas of caseous necrosis, and lymphocytes were much more plentiful both in the walls of the granulomata and in the interstitial infiltrates. With the other adjuvants, the granulomata consisted of a central clear area, in which the oil was located, surrounded by a dense layer of macrophages with numerous multinucleated giant cells and a narrower outer layer containing variable numbers of lymphocytes and plasma cells. The surrounding cellular infiltrate was composed of numerous vacuolated macrophages, giant cells, lymphocytes, plasma cells, and variable numbers of eosinophils. Small numbers of lymphocytic follicles or germinal centers were found in most cases.

DISCUSSION

Selective augmentation of the immune response to a wide range of antigens has been evaluated by using a variety of adjuvants (21).

TABLE 5. Results of parasitological examinations of blood samples collected after challenge

Treatment group	Mean prepatent period (days)	Proportion of cattle showing parasitemia
Immunized with VSSA and IFA	— ^a	0/5
Immunized with VSSA and CFA	5.0	2/4
Immunized with VSSA and MDP + IFA	—	0/5
Immunized with VSSA and poly(A-U) + IFA	5.0	2/5
Immunized with VSSA and <i>P. acnes</i>	4.25	4/4
Immunized with VSSA and <i>B. pertussis</i>	4.5	2/4
Immunized with VSSA and saponin	—	0/4
Nonimmunized	4.6	5/5

^a —, No cattle exhibited parasitemia.

Such studies have been carried out mainly on laboratory animals, as have investigations into the mode of action of different adjuvants (2, 3). Similar studies in commercial livestock are essential to determine the efficacy of adjuvants and their acceptability with regard to the effects on the quality of the carcass.

The present investigation has provided comparative information on the use of nine different adjuvants for the enhancement of the immune response to a soluble antigen in cattle; the antigen used was VSSA of *T. brucei*. It was found that marked differences existed in the ability of different adjuvants to potentiate both humoral and cell-mediated responses. The serological responses to the purified glycoprotein VSSA of immunized cattle as measured by the Farr assay indicated that the greatest specific responses were induced by the oil-based adjuvants (IFA, CFA, MDP, AHO, poly A-U) and saponin. The bacterial adjuvants (*P. acnes* and *B. pertussis*) and AH elicited antibodies the binding of which was intermediate between the ABCs of the former group and those resulting from the inoculation of antigen alone. A similar progression of results was obtained in a solid-phase radioimmunoassay (data not shown). The kinetics of the serological responses were comparable to those achieved with adjuvants and bacterial antigen in previous studies (20). From the data, CFA appeared to provide the greatest stimulus for antibody production in cattle; little difference was evident between the remaining four oil-based adjuvants and saponin.

The induction in immunized cattle of delayed-type hypersensitivity (DTH) as assessed by specific lymphocyte transformation in vitro was most apparent in animals inoculated with oil-based adjuvants and, to a lesser extent, *P. acnes*. Whereas saponin induced significant responses, it failed to stimulate DTH in vitro and ranked in this respect with *B. pertussis*, AH, and nonadjuvanted antigen at the levels of DTH in control cattle. On the basis of transformation, CFA and MDP appeared the most efficient adju-

vants. Moreover, the DTH responses in PBL from all cattle had declined toward that of control cattle by 6 to 7 weeks after inoculation. Infection of cattle did not boost the in vitro proliferative responses of PBL to specific antigen in this and a previous study (11), a finding which contrasts with the situation observed in sheep exposed to infection with *Pasteurella haemolytica* (19).

Cross (8), who initially purified and characterized the VSSA of *T. brucei*, was able to induce protective immunity in mice by administering 4 weekly doses of 100 µg of VSSA in CFA. In the present study, it has been established that cattle can be immunized with a single inoculum of 200 µg of VSSA when given with certain adjuvants. In the first experiment, none of the immunized cattle was protected against infective challenge, although there was a delay in onset of parasitemia. However, subsequent examination of IL-Tat 1.3 suggested that, with a challenge dose of 10⁵ *T. brucei* organisms, the cattle did not receive a homogeneous population consisting of a single variant of *T. brucei*. This contention is supported by the finding that in the second experiment, using an identical immunization schedule with VSSA in IFA, complete protection was achieved against a challenge dose of 10⁴ *T. brucei* ILTat 1.3.

On the basis of the serological results obtained by the Farr assay and the specific proliferative responses of cultures of lymphocytes, CFA proved to be the most successful of the adjuvants tested in the second experiment. However, two of the four cattle immunized with CFA were shown to be susceptible to challenge. The reasons for this discrepancy are unclear, since no differences in serum neutralizing activity were found between the protected and the susceptible cattle in the group. Although the levels of serum antibody induced by either VSSA in IFA or VSSA with MDP in IFA were inferior to those in cattle receiving CFA, complete protection was afforded in these two groups. There appeared to be no advantage in the addition of

either MDP or poly (A-U) to the antigen preparation in IFA. Confrontation with the ILTat 1.3 variable antigen type boosted the titers of specific antibody only in cattle which became parasitic after the challenge infection. This was probably due to the rapid elimination of the parasite in resistant cattle before sufficient numbers of trypanosomes to initiate an anamnestic antibody response were generated.

Saponin was the only non-oil-based adjuvant which induced a protective immune response, with serum antibody activity in the first 3 weeks after immunization approaching that observed in cattle given VSSA in IFA. This protective response was achieved in the absence of any detectable lymphocyte transformation response, thus indicating that the induction of a DTH-type response is not essential for protective immunity in trypanosomiasis. The success of saponin in immunization was further emphasized by the finding that it did not cause any detectable reaction at the site of injection. By contrast, reactions were found in all animals which received oil-containing adjuvants. Although in most instances these reactions were small, they would probably render the preparations unacceptable for use in meat-producing animals.

The results of this trial illustrated the overall effectiveness of saponin as an adjuvant in cattle for immunization against a soluble antigen (VSSA) where an antibody response appears to confer protection. Complete protection was achieved with a single immunizing inoculum which did not induce local reactions. The results were consistent with previous experiments in laboratory animals which suggested the value of saponin over CFA in enhancing the protective immune response to *T. congolense* in mice immunized with soluble antigen (12). It is hoped that the information generated in this study will be of value in selection of adjuvants for use in cattle, particularly where soluble protein antigens are involved. Further studies are required to compare the efficacy of adjuvants to boost humoral and cell-mediated immune responses to particulate and cell surface-associated antigens.

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